RESEARCH NOTE

Incidence of glycopeptide hetero-intermediate *Staphylococcus aureus* strains in Maltese hospitals

M. A. Borg[1], *R. Zerafa*[2], *D. Morrison*[3] *and P. Cuschieri*[2]

[1]Infection Control Unit and [2]Microbiology Laboratory, St Luke's Hospital, Guardamangia, Malta; [3]Scottish MRSA Reference Laboratory, Glasgow, UK

ABSTRACT

The incidence of hetero-intermediate glycopeptide susceptibility among *Staphylococcus aureus* isolates in Malta, a country with a high incidence of methicillin resistance, was studied by screening 454 non-repetitive *S. aureus* isolates on teicoplanin-supplemented agar plates, followed by Etests and genotypic studies. All strains were susceptible to vancomycin, but four (0.88%) exhibited teicoplanin MICs of >12 mg/L. High methicillin-resistant *S. aureus* endemicity was not an accurate predictor of the emergence of non-susceptibility to glycopeptides.

Keywords h-GISA, Malta, resistance, *Staphylococcus aureus*, teicoplanin, vancomycin

Original Submission: 12 November 2004; **Accepted:** 18 December 2004

Clin Microbiol Infect 2005; 11: 405–407
10.1111/j.1469-0691.2005.01110.x

Strains of *Staphylococcus aureus* with reduced susceptibility to the glycopeptides vancomycin and teicoplanin were first reported from Japan in 1997 [1]. Further reports from Japan have indicated that these strains of *S. aureus*, with vancomycin MICs of <4 mg/L, may include resistant sub-population variants, occurring at a frequency of approximately 1×10^{-6} [2]. The clinical significance of these hetero-resistant isolates remains a matter of considerable debate and it is not recommended to report them as glycopeptide-intermediate *S. aureus* (GISA) in clinical laboratory reports [3]. Nevertheless, strains of glycopeptide hetero-intermediate *S. aureus* (hGISA) have been associated with therapeutic failure [4]. It has also been postulated that hGISA strains may be precursors for the development of the GISA phenotype [2]. Therefore, their quantification may offer an insight into the possibility of emergence of GISA in a particular geographical location [5].

Malta is an island country in the Mediterranean with a population of 400 000, and is considered to have a high endemicity of methicillin-resistant *S. aureus* (MRSA), with >45% of *S. aureus* isolates from blood cultures being reported as methicillin-resistant. No clinical cases of GISA have ever been reported. Consecutive non-repetitive *S. aureus* isolates (n = 454) were examined over a 9-month period between January and September 2003 at the Microbiology Laboratory of St Luke's Hospital, the only tertiary-care hospital on the island. Glycopeptide susceptibility testing followed the technical procedure recommended by the European Antimicrobial Resistance Surveillance System [6], based on the study of Walsh *et al.* [7] and the method of screening for GISA adopted by the French Microbiology Society [8]. Screening was undertaken by growing the bacteria overnight in brain–heart infusion broth (Oxoid, Basingstoke, UK); 10 μL of the stationary-phase culture was then inoculated on to a Mueller–Hinton agar (Oxoid) plate containing teicoplanin 5 mg/L. The plates were incubated for 48 h at 37 ± 0.5°C. Growth of two or more colonies was deemed to be a positive result, whereupon the colonies were analysed further by the Etest method.

The inoculum for Etests was prepared in brain–heart infusion broth (BBL, Cockeysville, MD, USA) to a density of 2× McFarland standards. Aliquots of 200 μL were spread evenly with a swab on two 90-mm brain–heart infusion agar (Oxoid) plates. The plates were then left to stand to allow the inoculum to soak into the plate, after which Etest strips for teicoplanin and vancomycin were applied according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). The plates were read after 48 h at 37 ± 0.5°C, with

Corresponding author and reprint requests: M. A. Borg, Infection Control Unit, St Luke's Hospital, Guardamangia MSD 07, Malta
E-mail: michael.a.borg@gov.mt

Table 1. Isolates from Malta with the glycopeptide hetero-intermediate resistance phenotype (hGISA)

Source	Resistance profile (disk tests)	PFGE profile	Etest MIC (mg/L)	
			Vancomycin	Teicoplanin
Dialysis; tertiary hospital	PnMtCxErClGnCpKmStNoTb	Malta-1a	3	16
District general hospital	PnMtCxErClGnKmStTb	Malta-1b	4	12
Geriatric hospital	PnMtCxErClCpKmNoTb	Malta 2	3	12
Intensive care unit; tertiary hospital	PnMtFdTeKmStNo	Malta-3	3	16

PFGE, pulsed-field gel electrophoresis.
Pn, penicillin (1-μg disk); Mt, methicillin (5 μg); Cx, cefuroxime (30 μg); Er, erythromycin (5 μg); Cp, ciprofloxacin (1 μg); Cl, clindamycin (2 μg); Km, kanamycin (30 μg); Tb, tobramycin (10 μg); No, neomycin (10 μg); Fd, fusidic acid (10 μg); Te, tetracycline (10 μg); St, streptomycin (10 μg); Gn, gentamicin (10 μg).

particular attention paid to any 'micro' colonies or small colony variants growing in the Etest ellipse. If colonies were found growing at cut-off levels of ≥12 mg/L for the teicoplanin plate, or ≥8 mg/L for both the teicoplanin and vancomycin plates [7], the isolates were then analysed by pulsed-field gel electrophoresis typing and 16S–23S rRNA intergenic spacer polymorphism analysis [9,10].

Of the 454 isolates tested, 14 (3.1%) yielded an average of 24 CFU/plate (range: 2–100 CFU/plate) on the glycopeptide screening plate. Vancomycin MICs for these 14 isolates were 1.5–4 mg/L; however, only four (0.88%) isolates were defined as glycopeptide non-susceptible on the basis of a teicoplanin MIC of >12 mg/L. Two isolates had been submitted from within St Luke's Hospital itself (renal and intensive care units), while the other two originated from a geriatric rehabilitation hospital and a district general hospital, respectively (Table 1). All four of these isolates were non-susceptible to methicillin and penicillin. This is not surprising, as resistance to glycopeptides in *S. aureus* is found predominantly among MRSA isolates [11]. The isolate designated Malta-3 was also resistant to cefuroxime, erythromycin and ciprofloxacin. The three other isolates had wider antimicrobial resistance profiles, including resistance to lincomycins and aminoglycosides.

The four isolates were grouped into three clusters by pulsed-field gel electrophoresis. Malta-3 was a clonal variant (four bands difference) of the most prevalent community MRSA clone (MLST type 80) in Europe, and carried the PVL toxin gene associated with this clone (results not shown) [12]. The two variants (one band difference) of the Malta-1 clone were similar to one of the major international epidemic MRSA clonal complexes (CC5) by pulsed-field gel electrophoresis and 16S–23S rRNA intergenic spacer polymorphism analysis. The first Japanese GISA isolate and five of the USA GISA isolates also belonged to this clonal complex [13].

Malta is among the countries with the highest incidence of MRSA in Europe [6]. Studies in other Mediterranean countries with high MRSA levels have reported varying GISA and h-GISA rates, ranging from 65% in Spain [14] to 1.1% in Italy [11] and 0.6% in France [15]. The rate of 0.88% identified in Malta supports the hypothesis that the mere prevalence of methicillin resistance in a particular geographical area is not an accurate predictor of the likelihood of glycopeptide non-susceptibility.

The results of the present study differed from those reported in other Mediterranean countries, in that all teicoplanin hetero-intermediate strains isolated in Malta were susceptible to vancomycin. Exposure to glycopeptides is recognised as a risk factor for the emergence of resistance in *S. aureus* [16]. Teicoplanin is the most popular glycopeptide used in Malta, with annual defined daily doses more than five-fold greater than those for vancomycin. Further studies are needed to establish whether the predominant use of teicoplanin in a setting with a high MRSA prevalence and substantial glycopeptide consumption has any impact on reducing the emergence of vancomycin resistance in *S. aureus*.

REFERENCES

1. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997; **40**: 135–136.

2. Hiramatsu K, Aritaka N, Hanaki H *et al.* Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* 1997; **350**: 1670–1673.
3. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. *Emerg Infect Dis* 2001; **7**: 327–332.
4. Ploy MC, Grelaud C, Martin C, De Lumley L, Denis F. First clinical isolate of vancomycin-intermediate *Staphylococcus aureus* in a French hospital. *Lancet* 1998; **351**: 1212.
5. Johnson AP. Intermediate vancomycin resistance in *Staphylococcus aureus*: a major threat or a minor inconvenience. *J Antimicrob Chemother* 1998; **42**: 289–291.
6. European Antimicrobial Resistance Surveillance System. *Technical guide for the detection of VISA/VRSA.* Bilthoven: National Institute for Public Health and the Environment (RIVM), 2004. http://www.earss.rivm.nl.
7. Walsh TR, Bolmström A, Qwärnström A *et al.* Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. *J Clin Microbiol* 2001; **39**: 2439–2444.
8. Chesneau O, Morvan A, Solh NE. Retrospective screening for heterogeneous vancomycin resistance in diverse *Staphylococcus aureus* clones disseminated in French hospitals. *J Antimicrob Chemother* 2000; **45**: 887–890.
9. MacKenzie FM, Greig P, Morrison D, Edwards G, Gould IM. Identification and characterization of teicoplanin-intermediate *Staphylococcus aureus* blood culture isolates in NE Scotland. *J Antimicrob Chemother* 2002; **50**: 689–697.
10. Kumari DN, Keer V, Hawkey PM *et al.* Comparison and application of ribosome spacer DNA amplicon polymorphisms and pulsed-field gel electrophoresis for differentiation of methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* 1997; **35**: 881–885.
11. Marchese A, Balistreri G, Tonoli E, Debbia EA, Schito GC. Heterogeneous vancomycin resistance in methicillin-resistant *Staphylococcus aureus* strains isolated in a large Italian hospital. *J Clin Microbiol* 2000; **38**: 866–869.
12. Vandenesch F, Naimi T, Enright MC *et al.* Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton–Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003; **9**: 978–984.
13. Walsh TR, Howe RA. The prevalence and mechanisms of vancomycin resistance in *Staphylococcus aureus*. *Ann Rev Microbiol* 2002; **56**: 657–675.
14. Ariza J, Pujol M, Cabo J *et al.* Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. *Lancet* 1999; **353**: 1587–1588.
15. Reverdy ME, Jarraud S, Bobin-Dubreux S *et al.* Incidence of *Staphylococcus aureus* with reduced susceptibility to glycopeptides in two French hospitals. *Clin Microbiol Infect* 2001; **7**: 267–272.
16. Hageman JC, Pegues DA, Jepson C *et al.* Vancomycin-intermediate *Staphylococcus aureus* in a home health-care patient. *Emerg Infect Dis* 2001; **7**: 1023–1025.

RESEARCH NOTE

Microbiology of sinusitis and the predictive value of throat culture for the aetiology of sinusitis

A. Ilki[1], N. Ulger[1], S. Inanlı[2], E. Ozer[2], C. Arikan[3], M. Bakır[4] and G. Soyletir[1]

[1]Department of Microbiology, [2]Department of Otolaryngology, [3]Department of Paediatrics and [4]Division of Paediatric Infectious Disease, Marmara University, Istanbul, Turkey

ABSTRACT

A prospective study of throat cultures and maxillary sinus aspirates from children with chronic sinusitis (n = 21), acute sinusitis (n = 28) or a clinical diagnosis of chronic adenoiditis (n = 41) was performed. Seventy-two bacterial pathogens were isolated from sinus aspirates from 52% of the study population. *Haemophilus influenzae* was most common pathogen, followed by *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and group A streptococci. Quantitative throat cultures had positive predictive values of 41%, 53% and 75% for *H. influenzae*, *Strep. pneumoniae* and *M. catarrhalis*, respectively, while negative predictive values were 93–98%, indicating that these three pathogens do not cause sinusitis when absent from the throat.

Keywords Children, *Haemophilus influenzae*, *Moraxella catarrhalis*, sinusitis, *Streptococcus pneumoniae*, throat cultures

Original Submission: 9 May 2004; **Revised Submission:** 13 December 2004; **Accepted:** 23 December 2004

Clin Microbiol Infect 2005; 11: 407–410
10.1111/j.1469-0691.2005.01132.x

Sinusitis is a common complication of upper respiratory tract virus infection and allergic inflammation [1]. Sinus cavity aspiration is the most

Corresponding author and reprint requests: A. Ilki, Marmara University, Faculty of Medicine, Department of Microbiology, Tibbiye Cad. No. 49, 81326 Haydarpasa, Istanbul, Turkey
E-mail: ailki@superonline.com